Emergence of meropenem/vaborbactam resistance in KPC-producing *Klebsiella pneumoniae* causing bloodstream infections in northern Italy, 2018

Paolo Gaibani (✉ paolo.gaibani@unibo.it)

IRCCS S.Orsola-Malpighi <https://orcid.org/0000-0003-3791-8635>

Donatella Lombardo

IRCCS S.Orsola-Malpighi

Linda Bussini

IRCCS S.Orsola-Malpighi

Federica Bovo

IRCCS S.Orsola-Malpighi

Maddalena Giannella

IRCCS S.Orsola-Malpighi

Michele Bartoletti

IRCCS S.Orsola-Malpighi

Pierluigi Viale

IRCCS S.Orsola-Malpighi

Maria Carla Carla Re

IRCCS S.Orsola-Malpighi

Simone Ambretti

IRCCS S.Orsola-Malpighi

Research Article

Keywords: Meropenem/Vaborbactam, Ceftazidime/Avibactam, porins, combination treatment, cross-resistance, KPC

Posted Date: February 23rd, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-237255/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License. [Read Full License](#)

Abstract

We aimed to investigate the incidence of meropenem/vaborbactam-resistance among KPC-producing *Klebsiella pneumoniae* (KPC-Kp) bloodstream infection in a large Italian hospital. Meropenem/vaborbactam-resistance was found in 8% (n = 5) KPC-Kp, while 5% (n = 3) strains exhibited cross-resistance to ceftazidime/avibactam. Genomic analysis revealed that meropenem/vaborbactam-resistance was associated to non-functional OmpK35 and OmpK36 porins and no specific mutation was associated to cross-resistance. No specific antimicrobial treatment was related to favorable clinical outcome, while cross-resistance was not associated to higher clinical and/or microbiological failures. Our study indicated that resistance to meropenem/vaborbactam was due to porins deficiency and is associated to reduced susceptibility both to ceftazidime/avibactam and carbapenems.

Introduction

Management and treatment of patients with infections due to *Klebsiella pneumoniae* carbapenemase (KPC)-producing *K. pneumoniae* (KPC-Kp) is a daily challenge in clinical practice. In the last decade, the treatment of serious KPC-kp infections was frequently based on the combination of last-resort agents associated with nephrotoxicity, sub therapeutic concentrations, and/or intermediate *in vitro* activity due to limited options [1].

On 20th November 2018 European Medicines Agency (EMA) approved meropenem/vaborbactam (MEM/VAB) for clinical use in adult patients with difficult to treat carbapenem-resistant *Enterobacterales* (CRE) infections, satisfying an important medical issue linked to antibiotic-resistant *Enterobacterales* (<https://www.ema.europa.eu/en/medicines/human/EPAR/vaborem>). MEM/VAB represents a novel antibiotic combination therapy, approved for clinical use in Europe indicated for complicated urinary tract infection (cUTI), complicated intra-abdominal infections (cIAI), hospital-acquired pneumonia (HAP), ventilator-acquired pneumonia (VAP) and bacteremia associated with these infections [2].

In vitro studies demonstrated excellent activity of MEM/VAB against KPC-producers with a low ability to acquire resistance [3]. Recent clinical study showed that MEM/VAB had a superior rate of clinical cure against CRE infections in patients with bacteremia, compared to other available therapies [2]. However, limited information is still available on the efficacy of MEM/VAB in clinical practice and the *in vivo* evolution of emerging resistant strains [3]. Recently, *in vitro* studies demonstrated that mutations causing loss-of-function of OmpK35 and OmpK36 porins or reduced production can generate resistance to MEM/VAB and/or ceftazidime/avibactam (CAZ/AVI) [4, 5].

Aim of this study was to investigate the epidemiology of meropenem/vaborbactam resistance among KPC-Kp strains isolated from patients with bloodstream infection (BSI) and characterize genomically MEM/VAB-resistant strains.

Materials And Methods

Between 1st January to 31st December 2018, we collected all KPC-producing *K. pneumoniae* isolated from patients with BSIs hospitalized in the large tertiary-care university hospital in Bologna. The study was conducted in accordance with the Declaration of Helsinki. Analysis was performed with anonymized database. Bacteria was identified by MALDI-TOF (Bruker Daltonics, Germany) and antimicrobial susceptibility testing was performed by MicroScan Walkaway system and confirmed by MIC test strip (Liofilchem, Italy). The MIC value for colistin was determined by reference broth microdilution method [4]. MIC results were interpreted according to EUCAST clinical breakpoints v11.0 (http://www.eucast.org/clinical_breakpoints/). Carbapenemase production was determined by MALDI-TOF and/or NG-Test CARBA 5 (NG Biotech, France), as previously described [6]. Molecular assay was performed to identify carbapenemase gene (Xpert Carba-R, Cepheid).

Whole-genome sequencing of KPC-producing *K. pneumoniae* (KPC-Kp) strains was performed to identify the molecular mechanism at the basis of meropenem/vaborbactam-resistance as previous described [7]. Briefly, libraries were prepared by the Nextera DNA flex sample preparation kit and sequenced using the Illumina Iseq100 platform (Illumina, San Diego, USA) with a 2x150 paired end run. All read sets were evaluated by FastQC software and then assembled with SPAdes v.3.10 with careful settings. Assembled genomes were screened for Sequence type, known antimicrobial resistance, Sequence type (ST) by CGE server (<https://cge.cbs.dtu.dk/services/>). Porin genes and Tn4401 isoform were manually investigated by BLAST analysis. A core genome single nucleotide polymorphism (SNP) phylogeny was generated using Parsnp software [8] using draft genomes of KPC-producing *K. pneumoniae* CC258 strains isolated in Italy, as previously described [8]. Single nucleotide polymorphisms (SNPs) and insertion-deletions (Indels) between MEM/VAB-resistant KPC-Kp genomes were investigated using Breseq [9].

Clinical outcome was defined as all-cause mortality at day 30 after BSI onset (index BC collection day). Microbiologic failure was defined as isolation of KPC-Kp strain with similar antimicrobial susceptibility pattern following 30 days of target antimicrobial treatment.

Results

During the study period, *in vitro* activity of MEM/VAB has been evaluated against 62 KPC-Kp strains isolated from patients with BSIs. The KPC-Kp distribution revealed that 45.2% of bacteremic patients were hospitalized in intensive care units (ICUs), 34% in medicine, 9.7% in haemato-oncology, 6.4% in surgical and 4.7% in transplantation wards.

According to EUCAST breakpoints, 54 out of 62 strains (87%) were susceptible to meropenem/vaborbactam with a median MIC of 0.5 mg/L (interquartile range [IQR] 0.25-1). On opposite, eight KPC-Kp were resistant to meropenem/vaborbactam by exhibiting a median MIC of 256 mg/L (IQR 64–256), as shown in Table 1. Of note, three out of eight (37.5%) meropenem/vaborbactam-resistant KPC-Kp strains were also resistant to ceftazidime/avibactam (median MIC 32, IQR 32-256), while others meropenem/vaborbactam-resistant strains showed a CAZ/AVI MIC equal to 8 mg/L (Table 1).

Genomic analysis showed that meropenem/vaborbactam-resistant strains belonged to Clonal Complex 258 (ST1519, ST258 and ST512) and shared similar genetic resistance determinants responsible for resistance to β -lactams, aminoglycoside, fluoroquinolone and fosfomycin (Table 1). In particular, analysis of β -lactam resistance genes showed that all isolates shared wild-type *bla*_{KPC-3} gene, while 5 out of 8 carried *bla*_{SHV-182} variant. In addition, environment of *bla*_{KPC-3} gene demonstrated that all three KPC-Kp strains harbored Tn4401 isoform a. Genetic analysis of porin genes showed that all meropenem/vaborbactam-resistant KPC-Kp strains had truncated OmpK35 and the insertion of glycine and aspartic acid within OmpK36 at position 134-135 (GD134-135).

Based on genome alignment, a total of 234 SNPs in KpBO3, 336 in KpBO6, 137 in KpBO7, 137 in KpBO8, 115 in KpBO11, 111 in KpBO12, 156 in KpBO13 and 110 in KpBO14 were observed against genome of the KpBO3 strain as reference. Comparative analysis of single nucleotide polymorphisms (SNPs) and insertion-deletions (Indels) between MEM/VAB-resistant KPC-Kp strains showed that most SNPs were located intergenic regions or in hypothetical proteins and that no significant difference between MEM/VAB-resistant strains and cross-resistant (*i.e.* MEM/VAB and CAZ/AVI-resistant) KPC-Kp strains were observed in genes encoding antimicrobial resistance. In addition, phylogenetic analysis showed that genomes of KPC-Kp strains resistant to meropenem/vaborbactam clustered into a single clade (Figure S1, Supplementary data).

Retrospective clinical analysis showed that patients with BSI due to KPC-Kp resistant both to ceftazidime/avibactam and meropenem/vaborbactam had short time of CPE colonization (median 4 days, IQR 0-7). Clinical characteristics of patients with meropenem/vaborbactam-resistant KPC-Kp are shown in Table 2. The 30-days mortality rates were 100% (3/3) for patients with SOFA score between 10-20, 50% (1/2) for patients with score ranging from 5 to 10 and 0% (0/3) for patients below 4.

Clinical success was achieved in 50% (4/8) of patients. None of patients were treated with meropenem/vaborbactam-based treatment. Of note, clinical failure occurred for different antimicrobial treatments and in 33% (1/3) of meropenem/vaborbactam and ceftazidime/avibactam –resistant KPC-Kp strains. In particular, no relapsing infection was observed for all antimicrobial combination treatment. At the same time, microbiological failure was achieved in one patient treated with colistin-meropenem therapy due to persistent bacteremia.

Discussion

In this study, we evaluated the activities of meropenem/vaborbactam against 62 KPC-Kp clinical strains collected from bacteremic patients during 2018. Our findings showed that resistance to meropenem/vaborbactam emerged in 13% of KPC-Kp strains isolated from bloodstream infection. Of note, meropenem/vaborbactam resistance was associated to high level of MICs for ceftazidime/avibactam. In particular, cross-resistance to ceftazidime/avibactam and meropenem/vaborbactam was observed in three KPC-Kp strains which exhibited high levels of MICs for both ceftazidime/avibactam, meropenem/vaborbactam and carbapenems.

Recent study showed that the selection of KPC-Kp mutants with reduced susceptibility to meropenem-vaborbactam was due to porin mutations associated to increase in the *bla*_{KPC} gene copy number, whereas was not associated to mutations in the coding region of *bla*_{KPC} gene [10]. Our results are in agreement with these findings as we found that meropenem/vaborbactam-resistance was due to OmpK35 and OmpK36 porins depletion in our KPC-Kp strains and no specific mutations were observed in *bla*_{KPC} gene. At the same time, our results showed that no specific mutations were observed in KPC-Kp isolates resistant both to meropenem/vaborbactam and ceftazidime/avibactam in comparison to strains resistant to meropenem/vaborbactam alone. These results are in accordance with previous findings that showed as cross-resistance to meropenem/vaborbactam and ceftazidime/avibactam was due to loss-of-function mutations in the OmpK35 and OmpK36 porins which are also associated to elevated MICs for β -lactams [4,5].

We observed that patients with BSI due to KPC-Kp resistant both to meropenem/vaborbactam and ceftazidime/avibactam had short colonization time, thus suggesting that cross-resistance did not result from selection pressure due to prolonged antimicrobial therapy while can be plausible that patients acquired cross-resistant strains during hospitalization.

Overall clinical and microbiological failure was observed in two out of three patients with pneumonia, while no specific treatment was associated to clinical success. At the same time, microbiological failure was observed only in a colistin-meropenem combination treatment. Of note, cross-resistance to meropenem/vaborbactam and ceftazidime/avibactam was not associated to clinical or microbiological failure.

The study is limited by the small number of patients with BSI due to meropenem/vaborbactam-resistant strains treated with different combination treatments. Further study is mandatory to evaluate the clinical outcome of different antimicrobial therapies against BSI due meropenem/vaborbactam and/or ceftazidime/avibactam –resistant strains.

In conclusion, here we described the emergence of meropenem/vaborbactam resistance among KPC-Kp strains isolated from bacteremic patients. Further studies are planned to characterize specific-mutations emerging *in vivo* under different antimicrobial therapy and evaluate the *in vitro* activity of novel antimicrobials (*i.e.* cefiderocol, imipenem/cilastatin/relebactam) against meropenem/vaborbactam and/or ceftazidime/avibactam-resistant strains.

Declarations

Funding

This work was supported by Italian Ministry of Health (Ricerca Finalizzata, Giovani Ricercatori, GR-2018-12367572).

Conflicts of interest/Competing interests

The authors declare that they have no conflict of interest.

Availability of data and material

The data are available for scientists having interest.

Code availability

Not applicable

Ethics approval

The study was conducted in the context of normal clinical routine. The study was conducted in accordance with the Declaration of Helsinki. Samples were coded and analysis was performed with anonymized database.

Consent to participate

Not applicable

Consent for publication

Not applicable

References

1. Giacobbe DR, Maraolo AE, Viscoli C (2017) Pitfalls of defining combination therapy for carbapenem-resistant *Enterobacteriaceae* in observational studies. Eur J Clin Microbiol Infect Dis 36:1707–09. <https://doi.org/10.1007/s10096-017-3010-z>

2. Wunderink RG, Giamarellos-Bourboulis EJ, Rahav G, Mathers AJ, Bassetti M, Vazquez J, et al (2018) Effect and Safety of Meropenem-Vaborbactam versus Best-Available Therapy in Patients with Carbapenem-Resistant *Enterobacteriaceae* Infections: The TANGO II Randomized Clinical Trial. Infect Dis Ther 7:439-55. <https://doi.org/10.1007/s40121-018-0214-1>

3. Lapuebla A, Abdallah M, Olafisoye O, Cortes C, Urban C, Quale J, Landman D (2015) Activity of meropenem combined with RPX7009, a novel β -lactamase inhibitor, against Gram-negative clinical isolates in New York City. Antimicrob Agents Chemother 59:4856–60. <https://doi.org/10.1128/aac.00843-15>

4. Gaibani P, Re MC, Campoli C, Viale PL, Ambretti S (2020) Bloodstream infection caused by KPC-producing *Klebsiella pneumoniae* resistant to ceftazidime/avibactam: epidemiology and genomic characterization. Clin Microbiol Infect 26:516.e1-516.e4. <https://doi.org/10.1016/j.cmi.2019.11.011>

5. Dulyayangkul P, Wan Nur Ismah WAK, Douglas EJA, Avison MB (2020) Mutation of *kvrA* Causes OmpK35 and OmpK36 Porin Downregulation and Reduced Meropenem-Vaborbactam Susceptibility in KPC-Producing *Klebsiella pneumoniae*. Antimicrob Agents Chemother 64:e02208-19. <https://doi.org/10.1128/aac.02208-19>

6. Foschi C, Gaibani P, Lombardo D, Re MC, Ambretti S (2020) Rectal screening for carbapenemase-producing *Enterobacteriaceae*: a proposed workflow. J Glob Antimicrob Resist 21:86-90. <https://doi.org/10.1016/j.jgar.2019.10.012>

7. Gaibani P, Campoli C, Lewis RE, Volpe SL, Scaltriti E, Giannella M et al (2018) *In vivo* evolution of resistant subpopulations of KPC-producing *Klebsiella pneumoniae* during ceftazidime/avibactam treatment. J Antimicrob Chemother 73:1525-29. <https://doi.org/10.1093/jac/dky082>

8. Treangen TJ, Ondov BD, Koren S, Phillippy AM (2014) The Harvest suite for rapid core-genome alignment and visualization of thousands of intraspecific microbial genomes. Genome Biol 15:524. <https://doi.org/10.1186/s13059-014-0524-x>

9. Barrick JE, Colburn G, Deatherage DE, Traverse CC, Strand MD, Borges JJ et al (2014) Identifying structural variation in haploid microbial genomes from short-read resequencing data using breseq. BMC Genomics 15:1039. <https://doi.org/10.1186/1471-2164-15-1039>

10. Sun D, Rubio-Aparicio D, Nelson K, Dudley MN, Lomovskaya O (2017) Meropenem- Vaborbactam Resistance Selection, Resistance Prevention, and Molecular Mechanisms in Mutants of KPC-Producing *Klebsiella pneumoniae*. Antimicrob Agents Chemother 61:e01694-17. <https://doi.org/10.1128/aac.01694-17>

Tables

Table 1. Phenotypic and genotypic characteristics of meropenem/vaborbactam-resistant KPC-producing *Klebsiella pneumoniae* strains

Isolate	Patient	MIC (mg/L)				ST	Genetic determinants				Porins	
		MEM	CAZ/AVI	MEM/VAB	CST		Beta-lactams	Aminoglycoside	Fluoroquinolone	Sulfonamide	<i>OmpK35</i>	<i>O</i>
KpB03	1	256	32	256	0.25	512	<i>bla</i> _{KPC-3} , <i>bla</i> _{SHV-11}	<i>aac(6')-Ib</i>	<i>oqxA</i> , <i>oqxB</i> , <i>aac(6')Ib-cr</i>	<i>sul1</i>	truncated	G1:
KpB06	2	256	16	256	0.5	258	<i>bla</i> _{KPC-3} , <i>bla</i> _{SHV-12}	<i>aadA2</i> , <i>aph(3')-Ia</i> , <i>aac(6')Ib-cr</i>	<i>oqxA</i> , <i>oqxB</i> , <i>aac(6')Ib-cr</i>	<i>sul1</i>	truncated	G1:
KpB07	3	256	≥256	256	0.5	1519	<i>bla</i> _{KPC-3} , <i>bla</i> _{TEM-1A} , <i>bla</i> _{OXA-9} , <i>bla</i> _{SHV-11}	<i>aadA2</i> , <i>aph(3')-Ia</i> , <i>aac(6')Ib-cr</i>	<i>oqxA</i> , <i>oqxB</i> , <i>aac(6')Ib-cr</i>	<i>sul1</i>	truncated	G1:
KpB08	4	32	8	48	0.5	512	<i>bla</i> _{KPC-3} , <i>bla</i> _{SHV182} , <i>bla</i> _{TEM-1A} , <i>bla</i> _{OXA-9}	<i>aadA2</i> , <i>aph(3')-Ia</i> , <i>aac(6')-Ib</i>	<i>oqxA</i> , <i>oqxB</i> , <i>aac(6')Ib-cr</i>	<i>sul1</i>	truncated	G1:
KpB011	5	256	8	256	0.25	512	<i>bla</i> _{KPC-3} , <i>bla</i> _{SHV182}	<i>aadA2</i> , <i>aph(3')-Ia</i> , <i>aac(6')-Ib</i>	<i>oqxA</i> , <i>oqxB</i> , <i>aac(6')Ib-cr</i>	<i>sul1</i>	truncated	G1:
KpB012	6	256	8	256	0.5	512	<i>bla</i> _{KPC-3} , <i>bla</i> _{SHV182}	<i>aadA2</i> , <i>aph(3')-Ia</i> , <i>aac(6')-Ib</i>	<i>oqxB</i> , <i>oqxA</i> , <i>aac(6')Ib-cr</i>	<i>sul1</i>	truncated	G1:
KpB013	7	32	8	256	0.5	1519	<i>bla</i> _{KPC-3} , <i>bla</i> _{SHV182} , <i>bla</i> _{OXA-9}	<i>aadA2</i> , <i>aac(6')-Ib</i>	<i>oqxB</i> , <i>oqxA</i> , <i>aac(6')Ib-cr</i>	<i>sul1</i>	truncated	G1:
KpB014	8	256	8	256	0.25	512	<i>bla</i> _{KPC-3} , <i>bla</i> _{SHV182}	<i>aac(6')-Ib</i>	<i>oqxA</i> , <i>oqxB</i> , <i>aac(6')Ib-cr</i>	-	truncated	G1:

Abbreviations: MEM, meropenem; CAZ/AVI, ceftazidime/avibactam; MEM/VAB, meropenem/vaborbactam; CST, colistin; ST, sequence type.

Table 2. Clinical characteristics of patients with bloodstream infection due to Meropenem/Vaborbactam-resistant KPC-producing *Klebsiella pneumoniae* (KPC-Kp) strains

Patient	Isolate	Colonization days' prior infection	SOFA	Initial infection	Previous Treatment (days)	Time of isolation after initial treatment (days)	Antimicrobial combination therapy (days)	Risk factors	Clinical outcome at 30 days	Micro outcome at 30 days (days)
1	KpB03	0	13	Abdominal infection	Ceftazidime/Avibactam (12), Meropenem (32)	0	Meropenem-Colistin-Tigecycline (3)	-	Failure	NA
2	KpB06	4	2	Pneumonia	Meropenem-Colistin (27) Meropenem-Tigecycline (19)	0	Meropenem-Colistin (24)	-	Success	Successful
3	KpB07	7	1	CVC-related	Amoxicillin/Clavulanic acid (11)	1	Meropenem-Colistin (13)	CVVH	Success	Successful
4	KpB08	60	6	CVC-related	Oxacillin (11), Ertapenem (13)	0	Meropenem-Ceftazidime/Avibactam (24)	CKD	Success	Successful
5	KpB011	18	12	Pneumonia	Oxacillin (8), Piperacillin/Tazobactam (34)	15	Meropenem-Colistin (18)	-	Failure	Failed (15)
6	KpB012	3	20	Biliary infection	Piperacillin/Tazobactam (3) Meropenem-Tigecycline (3)	10	Meropenem-Tigecycline (1)	CKD, CVVH	Failure	NA
7	KpB013	5	5	Pneumonia	Piperacillin/Tazobactam (16) Meropenem-Tigecycline (29)	1	Meropenem-Ceftazidime/Avibactam (14)	CKD	Failure	NA
8	KpB014	60	3	Urinary infection	Amoxicillin/Clavulanic acid (20) Meropenem (19)	0	Meropenem-Ceftazidime/Avibactam (14)	CKD	Success	Successful

Abbreviations: Bloodstream infection, BSI; not changed, NC; Central Venous Catheter, CVC; Continuous Venous Hemofiltration, CVVH; Chronic Kidney Disease, CKD; not applicable, NA.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Supplementarydata.docx](#)